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Retinoblastoma Protein Expressed in Human Non-Hodgkin's Lymphoma Cells Generates Resistance Against Radiation-Induced Apoptosis

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Inactivation of the retinoblastoma susceptibility (Rb) gene has been observed in various cancers. Nevertheless, in several cancer cases, including non-Hodgkin's lymphoma, previous investigations showed that the Rb gene product (pRb) was abundantly expressed with varying degree. Here we report the THS-SP1.1 cell line isolated from non-Hodgkin's lymphoma. The THS-SP1.1 cells abundantly expressed pRb and showed resistance against radiation-induced apoptosis. Culture with the antisense phosphorothioate oligonucleotide complementary to the Rb gene augmented the apoptosis of THS-SP1.1 cells after radiation, whereas the control oligonucleotides did not. These data showed that pRb abundantly expressed in the lymphoma cells inhibited radiation-induced apoptosis. Am J. Hematol. 55:46–48, 1997. © 1997 Wiley-Liss, Inc.

Key words: retinoblastoma protein; non-Hodgkin's lymphoma; apoptosis

INTRODUCTION

In various cancers, the retinoblastoma susceptibility (Rb) gene was frequently inactivated. Nevertheless, the involvement of high levels of Rb gene product (pRb) expression in vivo was found in several tumors, including colorectal cancers [1,2], acute myelogenous leukemia [3], and non-Hodgkin's lymphoma (NHL) [4,5], although the expression was with varying fractions of pRb-positive cells present. In the present study, we isolated THS-SP1.1 cells from a patient with NHL and showed that the pRb expressed in the THS-SP1.1 cells possesses an inhibitory function for the induction of apoptosis.

MATERIALS AND METHODS

Cell Culture and Induction of Apoptosis

From the pleural effusion of a patient with non-Hodgkin's lymphoma (T-cell lymphoblastic type), we previously established a lymphoma THS–IV cell line [6]. Here we isolated a subclone, THS-SP1.1, after culture in 0.5% low-temperature melting agar (Sigma, St. Louis, MO). To induce the apoptosis, the cultured cells were irradiated. The proportion of live cells was determined by staining dead cells with 0.02% erythrosine B [6]. Apoptotic cells were determined by staining with 10 μM bisbenzimide Hoechst 33342 fluorochrome (Calbiochem, San Diego, CA).

Culture With Oligonucleotides

Twenty-one base oligonucleotides used for culture were synthesized using a 392 DNA synthesizer (Applied Biosystems, Foster City, CA) with phosphorothioate substitution at each base. The sequences of the oligonucleotides were those described in a previous report [7]. The cells were refed with fresh medium containing 7 μM oligonucleotides every day. Experiments were performed

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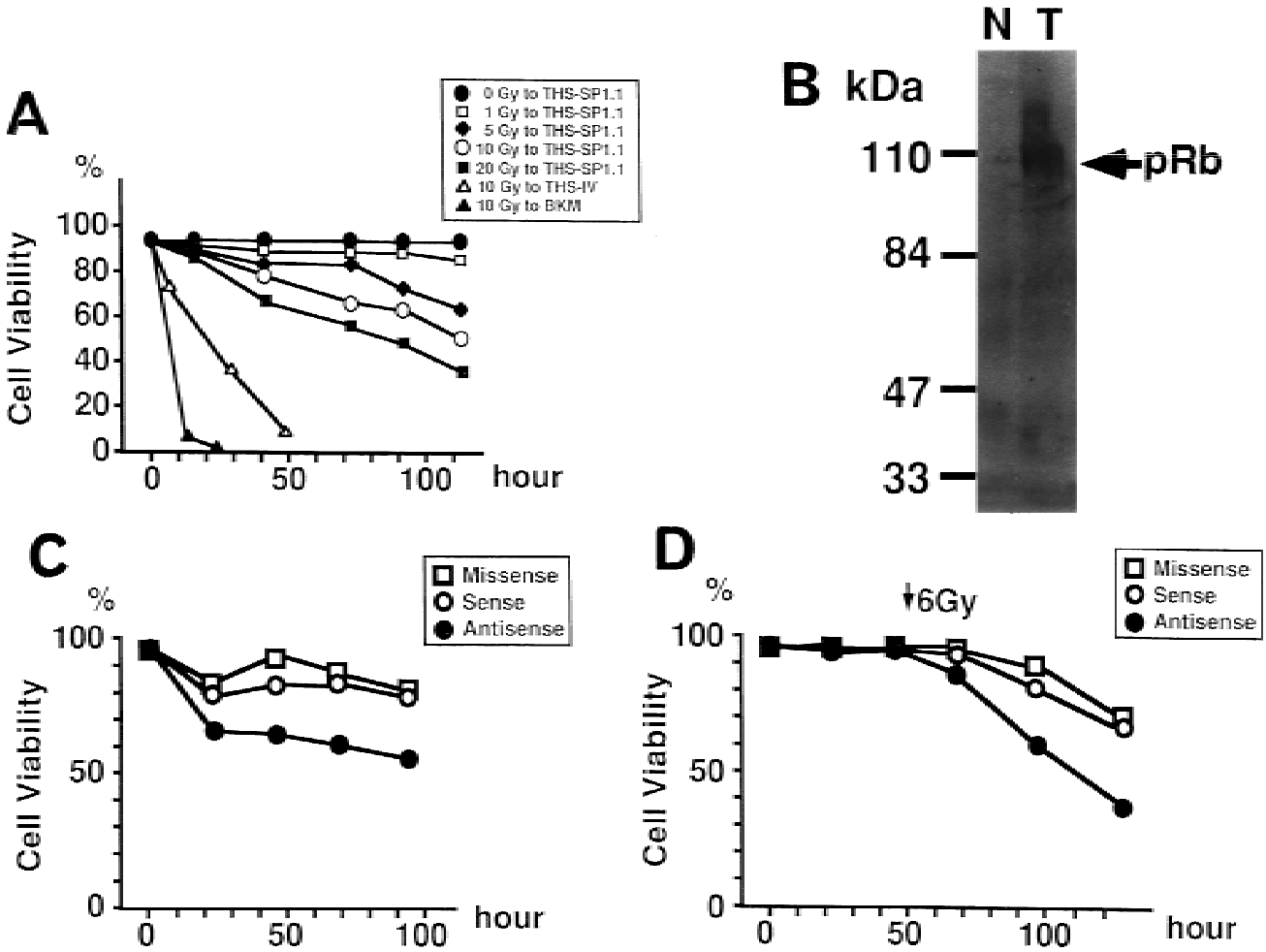

Fig. 1. A: **Cell viability of human lymphoma cell lines isolated from NHL after radiation. The viable cells was determined by erythrosine B staining. BKM cells are derived from B-cell lymphoma [6].** B: **Immunoblot analysis of pRb from THS-SP-1.1 cells. Thirty-μg of cell lysates was probed with anti-pRb monoclonal antobody (G3-245). N, normal lymphocytes; T, THS-SP1.1 cells.** C, D: **Enhancement of apoptosis of THS-SP1.1 cells by antisense oligonucleotide to the Rb gene. Cells were cultured in medium with 1.5% (C) or 10% FCS (D).** D: **Cells were exposed to 6-Gy radiation at 50 hr after culture (*arrow*).**

using the duplicated samples three times, and the reproducible data were shown.

DNA Blot and Protein Analysis

DNAs were extracted from cells and analyzed, and immunoblot analysis was performed, as described elsewhere [8].

RESULTS

Induction of Apoptosis

After radiation, cell counting of live THS-IV cells by excluding dead cells by erythrosine B staining showed that the proportion of the live cells was decreased to <10% of the total cells (Fig. 1A). The cell death of THS-IV, as determined morphologically, was apoptosis [6].

The subclone, THS-SP1.1, died slowly after radiation, taking a longer period of time than THS-IV and BKM cells [6] (Fig. 1A). Staining with the reagent Hoechst 33342 demonstrated that the dead THS-SP1.1 cells showed chromatin condensation, characteristic of apoptosis.

Analysis of THS-SP1.1 Cells

Immunoblot analysis revealed that the THS-SP1.1 cells expressed pRb much more abundantly than normal lymphocytes did (Fig. 1B), whereas DNA blot analysis did not indicate any alterations including rearrangements or amplifications of the Rb gene (data not shown). DNA blot and sequence analyses also indicated no alterations in the p53 gene (data not shown).

Culture With the Antisense Oligonucleotide

The culture in the medium with 1.5% fetal calf serum (FCS) containing 7 μM antisense oligonucleotide to the Rb gene increased cell death to approximately 50% of the

THS-SP1.1 cells after 100 hr, which was confirmed to be the apoptosis by Hoechst 33342 staining, whereas the culture with the sense oligonucleotide or the missense oligonucleotide did not (Fig. 1C). Moreover, the culture in the medium with 10% FCS containing antisense oligonucleotide to the Rb gene did not produce apoptosis, but after 3- or 6-Gy radiation, the proportion of the cell death substantially increased (Fig. 1D). Hoechst 33342 staining confirmed that the cell death induced by the antisense oligonucleotide and radiation was apoptosis.

DISCUSSION

We showed that THS-SP1.1 cells abundantly expressed pRb and that they were resistant against radiation-induced apoptosis. DNA blot analysis of the THS-SP1.1 cells did not detect any alterations in the Rb gene. Recent reports showed that the transfected Rb product (pRb) suppressed the apoptosis of osteosarcoma cells [9] and that the excess expression of the transfected Rb gene can overcome the p53-mediated apoptosis of HeLa cells [10]. These data showed that pRb abundantly expressed in the lymphoma cells may possess some inhibitory function against radiation-induced apoptosis, suggesting not only that inactivation of pRb enhanced cell growth of the tumor, but also that the deregulated presence of pRb might generate resistance against apoptosis of tumor cells.

Sequence analysis of the THS-SP1.1 cells did not detect any alterations in the p53 gene. Immunoblot analysis revealed that radiation induced the expression of the cdk-inhibitor p21/WAF1 in the THS-SP1.1 cells (data not shown). Thus, these data indicated that p53 was not inactivated in the THS-SP1.1 cells.

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